Appendix A

Specification Amendments with Notations to Indicate Changes Made

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For: SOMATOSTATINS AND METHODS

Deletions are bracketed, additions are underlined, and all changes are highlighed in gray.

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A two-phase rapid amplification of cDNA ends (RACE) PCR-based approach (Fig. 4) was used for the isolation and characterization of selected cDNA sequences as described previously (Moore et al., Gen. Comp. Endocrinol., 98, 253-261 (1995). In phase I, endogenous poly-A RNA was reverse transcribed from 15 μg of trout pancreatic total RNA with Superscript II reverse transcriptase (Gibco/BRL, Gaithersburg, MD) and a 37 nucleotide antisense adapter primer 5'-GGCCACGCGTCGACTAGTAC(T)[17]$_{17}$-3' (SEQ ID NO:22) (Gibco/BRL). Five microliters of the reverse transcription reaction were used as template for 3'-RACE PCR with a 21-base somatostatin gene-specific primer 5'-AAGAACTTCTTCTGGAAGAC-3' (GSP-1; SEQ ID NO:25) and the universal amplification primer [5'-CUACUACUACUAGGCCACGCGTCGACTAGT AC-3'] 5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3'(UAP; SEQ ID NO:23). After an initial denaturation cycle of 94°C for 5 minutes, 35 PCR cycles were performed, each consisting of 1-minute annealing (42°C), 1-minute extension (72°C), and 1-minute denaturation (94°C). In the last cycle, the extension time was increased to 10 minutes to ensure complete extension. The resulting PCR product (350 bp) was identified by electrophoresis on an agarose gel containing 1% (w/v) agarose (Gibco/BRL) and 1% (w/v) NuSeive GTG agarose (FMC Bioproducts, Rockland, ME) in 1X TBE Buffer, followed by ethidium bromide staining and UV transillumination. Amplified fragments were directly cloned into the TA cloning vector *PCR 2000* (Invitrogen, San Diego, CA). Positive colonies were identified by agarose gel electrophoresis of restriction enzyme digests (*Eco*RI; Promega, Madison, WI) of purified plasmid preparations (Del Sal et al., BioTech., 7, 514-519 (1989)). One to 2 μg of plasmid were

denatured and sequenced by the dideoxy chain-termination method (Sequenase Kit; U.S. Biochemicals Corp., Cleveland, OH) according to the manufacturer's protocol. All sequences were confirmed by sequencing multiple colonies from at least three independent PCR reactions and with two or more different primers in both directions, with dGTP didoexy nucleotides. Sequencing gels were made with 30% formamide to eliminate the possibility of G/C compressions.

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The results, shown in Fig. 8, indicate that the human somatostatin receptor type 1 has a greater affinity for salmonid SS-25 (SEQ ID NO:16) than for either mammalian SS-14 (SEQ ID NO:1) or mammalian SS-28 (SEQ ID NO:[53]21).